Role of Elm1 in cell wall integrity and virulence of *Candida glabrata*

Ito Y^{1,2)}, Miyazaki T^{1,3)}, Nakayama H⁴⁾, Morita A⁴⁾, Tanaka Y⁵⁾, Takazono T¹⁾, Saijo T¹⁾, Shimamura S¹⁾, Yamamoto K¹⁾, Imamura Y¹⁾, Izumikawa K³⁾, Yanagihara K⁶⁾, Kohno S¹⁾, Mukae H^{1,2)}

- 1) Second Department of Internal Medicine, Nagasaki University Hospital
- 2) Department of Respiratory Medicine, Nagasaki University Graduate School of Biomedical Sciences
- 3) Department of Infectious Diseases, Nagasaki University Graduate School of Biomedical Sciences
- 4) Faculty of Pharmaceutical Sciences, Suzuka University of Medical Science
- 5) Department of Infection and Host Defense, Tohoku Medical and Pharmaceutical University
- 6) Department of Laboratory Medicine, Nagasaki University Hospital

Correspondence :
Yuya Ito, MD
E-mail : y-ito@nagasaki-u.ac.jp

Introduction

The calcineurin signaling pathway plays critical roles in antifungal resistance and virulence in pathogenic fungi and has attracted attention as a novel target for antifungal therapy (1). The serine/threonine protein kinase Elm1 is regulated by calcineurin and involved in multiple cellular functions, including mitosis, cytokinesis, and morphogenesis in *Saccharomyces cerevisiae* (2, 3). In this study, we performed functional analysis of Elm1 in the clinically important fungal pathogen *Candida glabrata*.

Methods

The deletion and reintroduction of *ELM1* was performed in *C. glabrata* as described previously (1). The major cell wall components were measured using alkali-insoluble cell wall fraction (4). Antifungal susceptibility was examined using a commercially prepared colorimetric panel, Sensititre YeastOne microtiter panel. Virulence of the $\Delta elm1$ strain was examined using *Galleria mellonella* infection model (5). In this model, *C. glabrata* cells (2.0×10^6 cells / larva) were injected into the hemocoel through the last left pro-leg of larvae and survival was monitored daily for 7 days.

Results

Fig 1. Cell morphology The $\Delta elm1$ strain displayed elongated morphology.

Fig 2. Cell wall thickness and component content The $\Delta elm1$ strain had significantly thicker cell wall and increased chitin content compared with the wild-type and *ELM1*-reconstituted strains. (* $p < 0.01$ one-way ANOVA)

Table 1. Antifungal susceptibility

Strain	MIC (μg/ml)				
	AND	MCF	CPF	VRC	AMB
WT	0.12	0.03	0.12	0.25	0.5
$\Delta elm1$	0.12	0.015	0.06	0.25	0.25
$\Delta elm1 + ELM1$	0.12	0.03	0.12	0.25	0.5

AND, anidulafungin; MCF, micafungin; CPF, caspofungin
VRC, voriconazole; AMB, amphotericin B

Fig.3 Spot dilution assay

The $\Delta elm1$ strain increased sensitivity to high temperature and cell wall damaging agents including echinocandins, Congo red (CR), and calcofluor white (CFW).

Fig 4. Growth curve at 37°C The $\Delta elm1$ strain showed a decreased growth rate.

Fig 5. Survival curve of *G. mellonella* infected with *C. glabrata*.

The $\Delta elm1$ strain was more virulent than the wild-type strain. (* $p < 0.01$ Log-rank (Mantel-Cox) Test)

Conclusions

- The loss of Elm1 in *C. glabrata* resulted in elongated cell morphology, thicker cell wall with increased chitin content, decreased growth capacity, and increased sensitivity to cell wall stresses *in vitro*, however, the $\Delta elm1$ strain was more virulent than wild-type *in vivo*.
- To understand molecular basis of these phenomena, further analyses using an *elm1* strain controlled by the tetracycline-regulatable promoter and an *elm1* kinase-dead mutant are now in progress.

References

- 1) Miyazaki T, et al. Antimicrob Agents Chemotherapy 54: 1639-43, 2010.
- 2) Goldman A, et al. Mol Cell 55: 422-35, 2014.
- 3) Sreenivasan A, et al. Mol Cell Biol 19: 7983-94, 1999.
- 4) Tanaka Y, et al. PLoS One 11: e0161371, 2016.
- 5) Ames L, et al. Virulence 8: 1909-17, 2017.